Scaling pain threshold with microRNAs

Tuning pain with a defined microRNA cluster

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Pain is not something universally enjoyed, especially chronic pain involving nerve damage, referred to as neuropathic pain (***1***). Pain perception can be modulated in a variety of ways—for example, by focusing attention on the painful stimulus. On page 1168 of this issue, Peng *et al.*(***2***) report that pain threshold in dorsal root ganglion neurons (DRG), which relay peripheral sensory information to the central nervous system, can be modulated by mechanisms involving a specific cluster of microRNAs (miRNAs). Remarkably, the same cluster also regulates the threshold of neuropathic pain in DRG neurons that would not be involved in pain under normal conditions. The study provides important new insights into molecular mechanisms that control pain threshold, both in normal and pathological conditions.

Among the multitude of miRNAs expressed in the nervous system, the miR-183/96/182 miRNA cluster (henceforth, miR-183 cluster) is expressed in DRG (***3***). The cluster is also found in a few other sensory organs. In addition, DRG expression of miR-183 is reduced in models of chronic pain (***4***–***6***). Experimentally, the key feature of the study by Peng *et al.* is the generation of a mouse line that permits the selective excision of the miR-183 cluster. This line was crossed with other mouse strains, allowing the conditional elimination of the cluster either in all DRG neurons, or in a subpopulation of DRG pain-responsive neurons (so-called nociceptors), or in another subpopulation of DRG neurons exquisitely sensitive to touch, called low-threshold mechanoreceptors. Through free nerve endings in the skin, nociceptors detect potentially harmful stimuli like heat or noxious chemicals. These endings have a high threshold for mechanical stimuli, whereas mechanoreceptors respond to light touch, in particular through specialized endings that wrap hair follicles (***7***). In normal animals, sensitivity to mechanical but not to other stimuli was markedly increased when the miR-183 cluster was deleted from nociceptors, suggesting that these mi RNAs tune mechanical sensitivity. In an established model of neuropathic pain generated by a peripheral nerve lesion, the authors showed that deletion of the same miRNA cluster in mechanoreceptors markedly increased the response to mechanical stimuli. This increased sensitivity is thought to form the basis of chronic pain seen in neuropathic pain states through a circuitry involving the spinal cord (***1***), where mechanoreceptors make contact in more-anterior layers compared to nociceptors (see the figure).

An attractive feature of miRNAs is that they typically have multiple targets, such that entire pathways can be modulated. At the same time, this feature also makes the results of miRNA overexpression paradigms difficult to interpret, hence the value of the approach used by Peng *et al.* In particular, it allows meaningful transcriptional analyses to be performed using RNA extracted from the DRG after depletion of the miR-183 cluster. In control animals, a modest total of about 30 genes were dysregulated. Among the most immediately attractive transcripts from a functional standpoint were those encoding subunits α2δ associated with voltage-activated

calcium channels CACNA2D1 and CACNA2D2. Voltage-activated calcium channels are critical for the transmission of painful stimuli, and their associated subunits facilitate channel transport to the cell surface (***8***). Remarkably, these channels are targets of the calcium-channel inhibitor gabapentin, one of the few available drugs widely used in humans to treat neuropathic pain. So the new results by Peng *et al.* integrate well with what is already known in the field, with the crucial addition that the expression levels of functionally relevant components are now known to be regulated by the miR-183 cluster expressed in DRG sensory neurons. Indeed, Peng *et al.* demonstrate that this cluster is not expressed in appreciable amounts in the spinal cord, either during development or in the adult. These findings are all the more relevant in that they also seem to apply to humans: Indeed, analyses of RNA transcripts extracted from the DRG of more than 200 humans revealed an inverse correlation between the levels of the miR-183 cluster and those of channel and channel-associated proteins involved in setting pain threshold.

What about the changes observed in neuropathic pain? In animal models of neuropathic pain, expression of miR-183 in the DRG is decreased (***4***–***6***), a result confirmed and extended by the study of Peng *et al.*, which reports that the miR-183 cluster targets the vast majority of pain-regulated genes in mice with neuropathic pain, including *Cacna2d1* and *Cacna2d2*. This led the authors to test whether gabapentin reverses the intensified neuropathic pain phenotype in miR-183 cluster-deficient mice, which indeed turned out to be the case. In addition, with the knowledge that touch-sensitive mechanoreceptors are a major cellular source of problems in neuropathic pain, Peng *et al.* selectively activated these neurons using optogenetics. In control animals, this maneuver did not cause pain. By contrast, in the animal model of neuropathic pain, light activation of the same subpopulation of neurons increased pain-related behaviors, which could be attenuated by the administration of gabapentin.

The study by Peng *et al.* offers a remarkably detailed and specific molecular analysis of pain-related mechanisms taking place in the DRG. It provides new insights into regulatory changes occurring in DRG related to the detection of external stimuli and the changes following peripheral nerve lesion. An immediate question for future experiments is what regulates expression of the miR-183 cluster. Peng *et al.* report that expression of the miR-183 cluster is substantially higher during early mouse development than in the adult. These levels are decreased in neuropathic pain, but little is known about the mechanisms regulating expression of the miR-183 cluster in either situation. In addition, the results demonstrating an inverse correlation between the abundance of the miR-183 cluster and a number of mRNA transcripts in human DRG offer new possibilities for investigating the nature of genetic predisposition to pain. Although this is widely appreciated to be the case (***1***), there is still very little knowledge about gene polymorphisms or other changes, such as copy-number variations, associated with pain. Correlating the expression levels of relevant DRG transcripts with genome-wide polymorphism analyses may help to generate new knowledge in this area that in turn may lead to new treatments of conditions affecting tens of millions of people worldwide.

References

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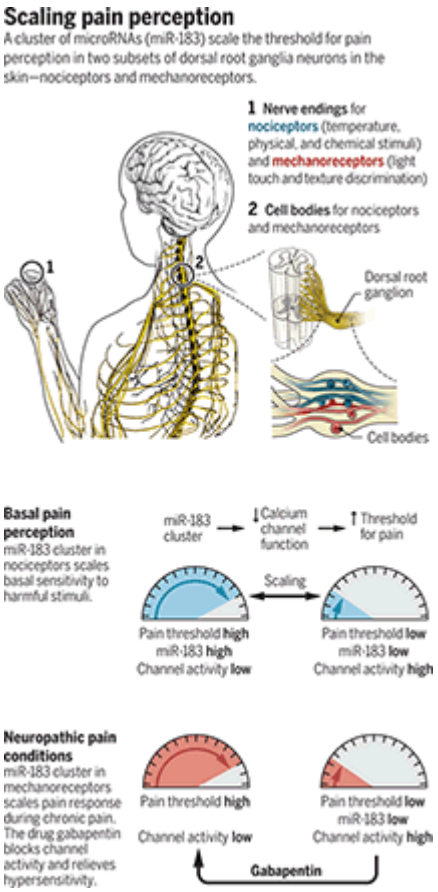
Figure



Scaling pain perception

A cluster of microRNAs (miR-183) scale the threshold for pain perception in two subsets of dorsal root ganglia neurons in the skin—nociceptors and mechanoreceptors.